Figure 1

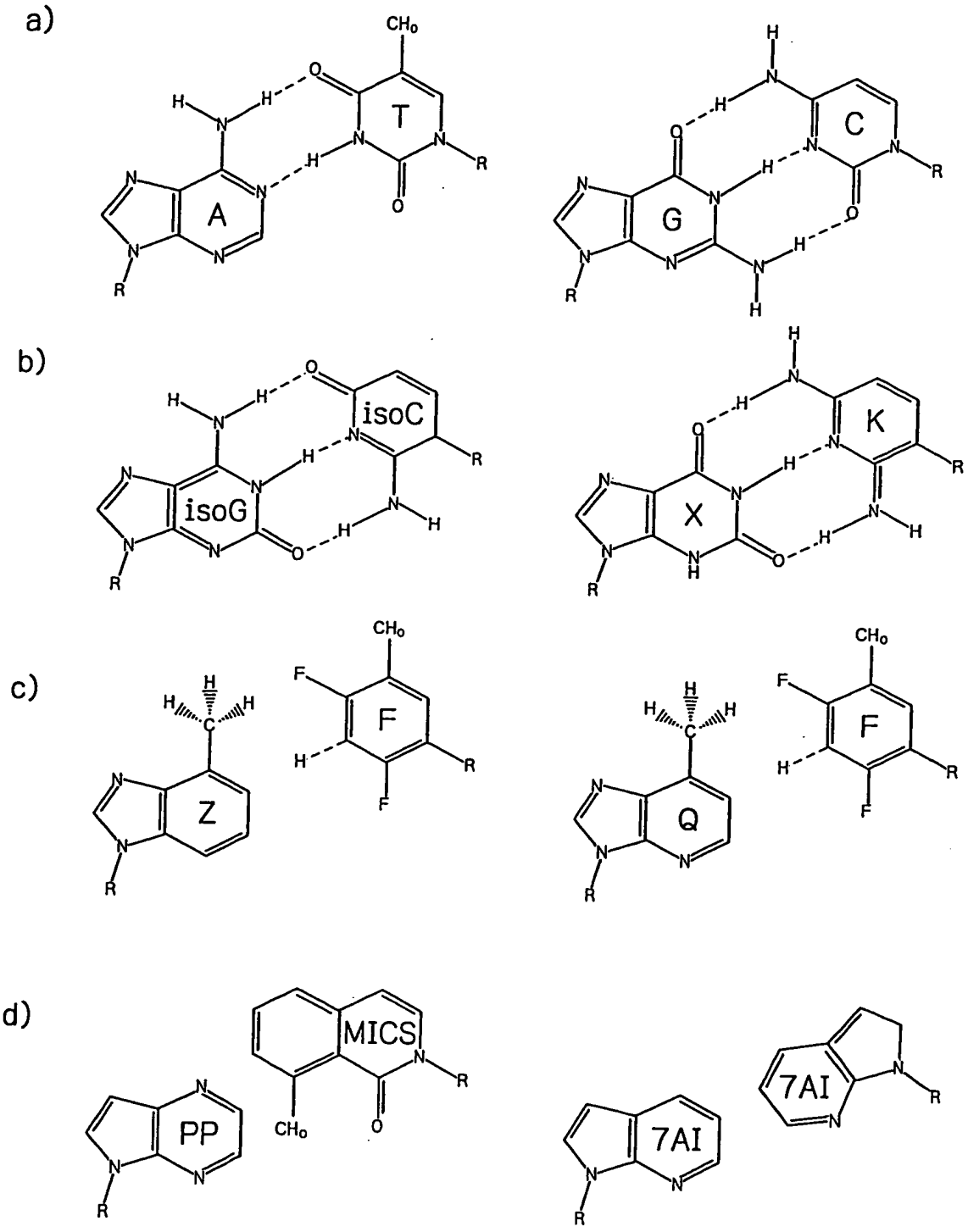
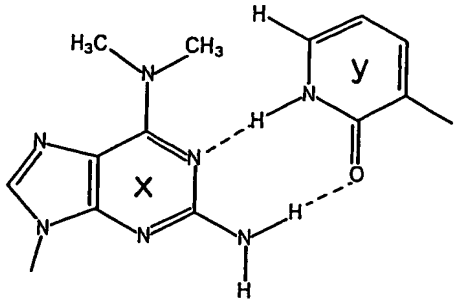
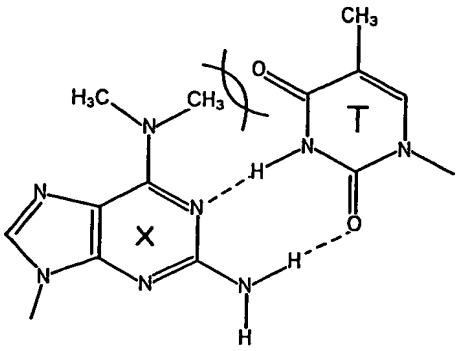


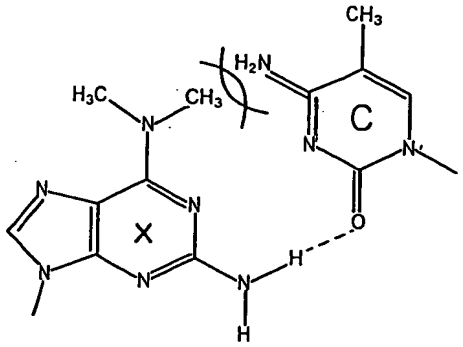
Figure 2

a)

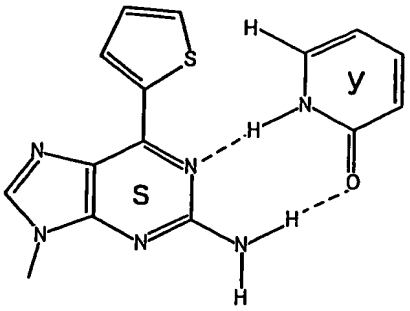


b)

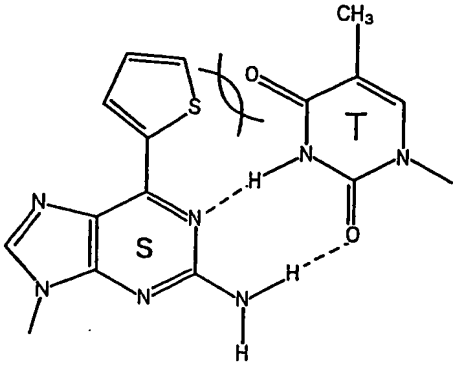




c)



d)



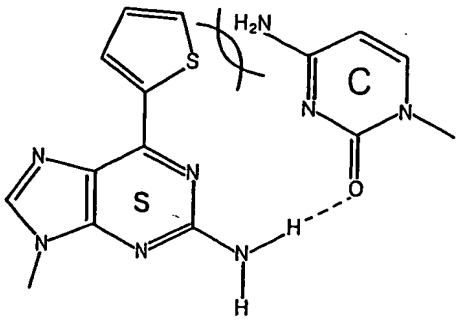
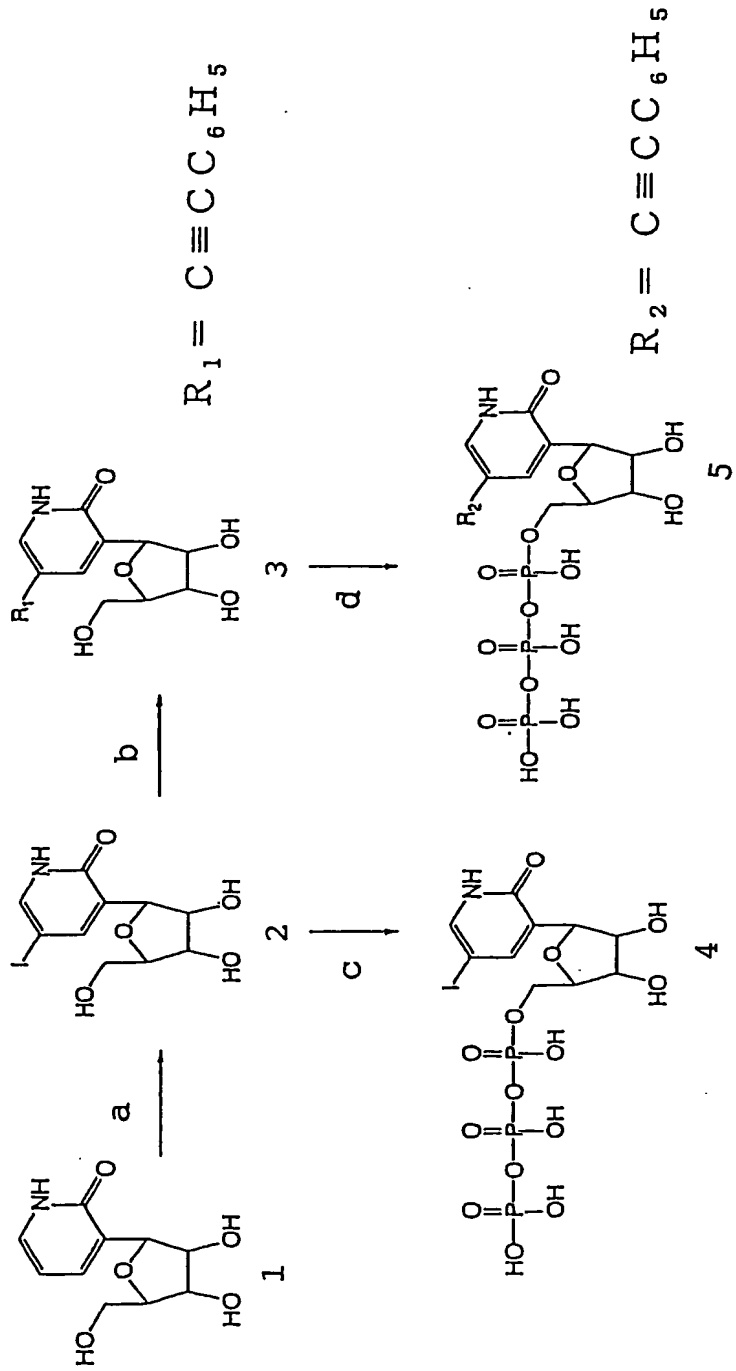


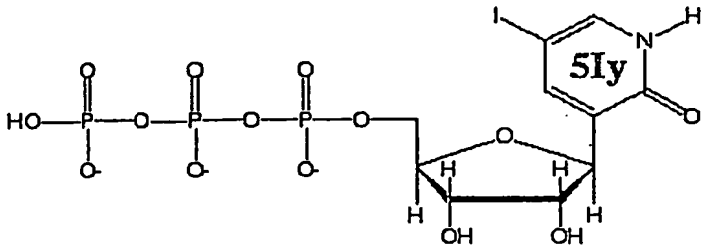
Figure 3



(a) I_2, KI, Na_2CO_3, 100°C, 4h. (b) $CF_3CONHCH_2CCH$, $Pd(Ph_3P)_4$, CuI, Et_3N, DMF, rt, 4-6h.
(c) (1) $POCl_3$, $(CH_3O)_3PO$, 0°C, 2h. (2) (n-$Bu_3NH)_2P_2O_7$, 0°C, 10min. (d) (1) $POCl_3$, 1,8-bis(dimethylamino)naphthalene, $(CH_3O)_3PO$, 0°C, 2h. (2) (n-$Bu_3NH)_2P_2O_7$, 0°C, 10min.
(3) conc. NH_4OH, rt, 10h.

Figure 4

a)


5Iy
HO
O
P
H
OH

b)

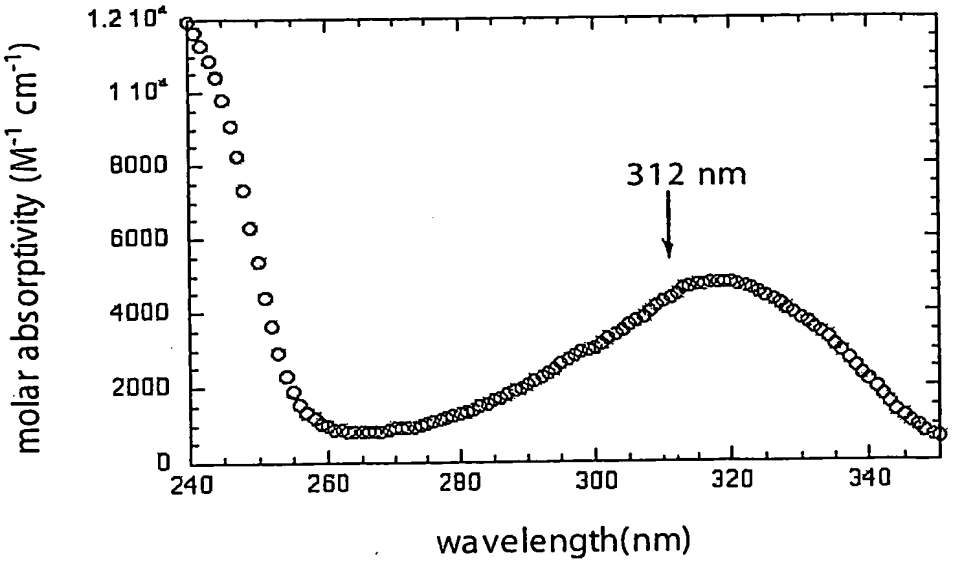

molar absorptivity (M^{-1} cm^{-1})
1.2 10⁴
1 10⁴
8000
6000
4000
2000
0
240
260
280
300
320
340
312 nm
wavelength(nm)

Figure 5

a)

5′-end primer; 39-mer

39.45 : 5′-GGTAATACGACTCACTATAGGGAGTGGAGGAATTCATCG

3′-end primer; 29-mer

29.45 :5′-GCAGAAGCTTGCTGTCGCTAAGGCATATG

29.45s84 :5′-GCAGAAGCTTGCTGTCsCTAAGGCATATG

29.45s87 :5′-GCAGAAGCTTGCTsTCGCTAAGGCATATG

29. 45s92 :5′-GCAGAAGCsTGCTGTCGCTAAGGCATATG

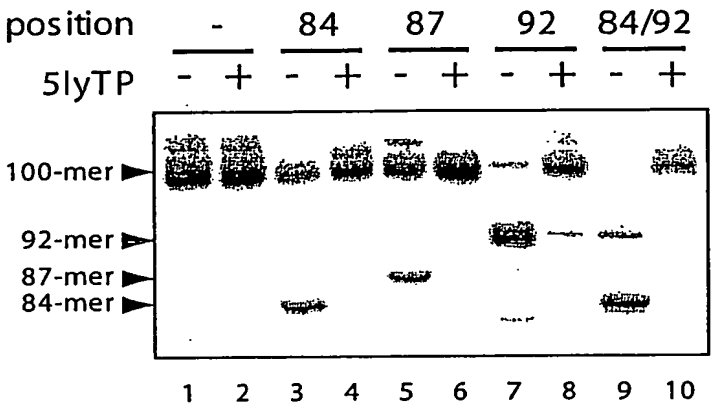
29.45s84/92 :5′-GCAGAAGCsTGCTGTCsCTAAGGCATATG

b)

5′- GGGAGUGGAG GAAUUCAUCG AGGCAUAUGU CGACUCCGUC UUCCUUCAAA
CCAGUUAUAA AUUGGUUUUA GCAUAUGCCU UAGCGACAGC AAGCUUCUGC

Figure 6

a)


position
-
84
87
92
84/92
5IyTP
- + - + - + - + - +
100-mer
92-mer
87-mer
84-mer
1 2 3 4 5 6 7 8 9 10

b)

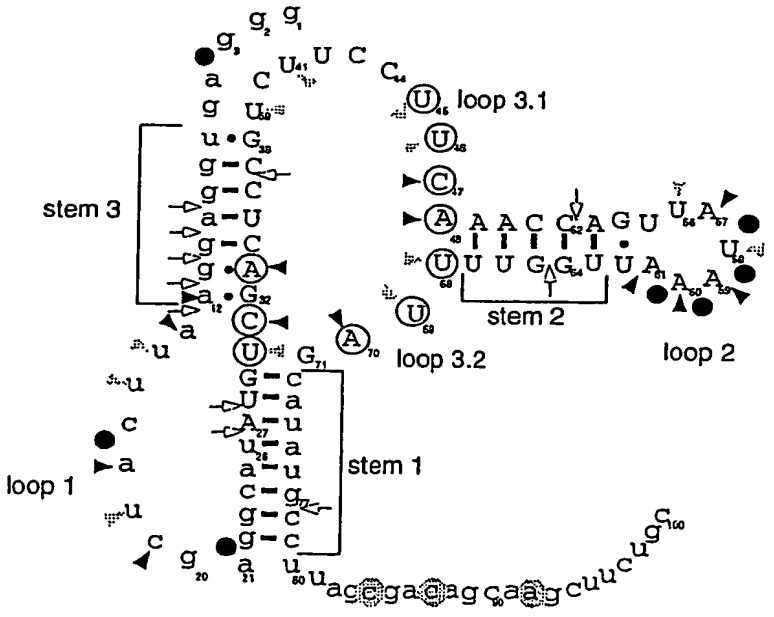

stem 3
loop 3.1
stem 2
loop 2
loop 3.2
stem 1
loop 1

Figure 7

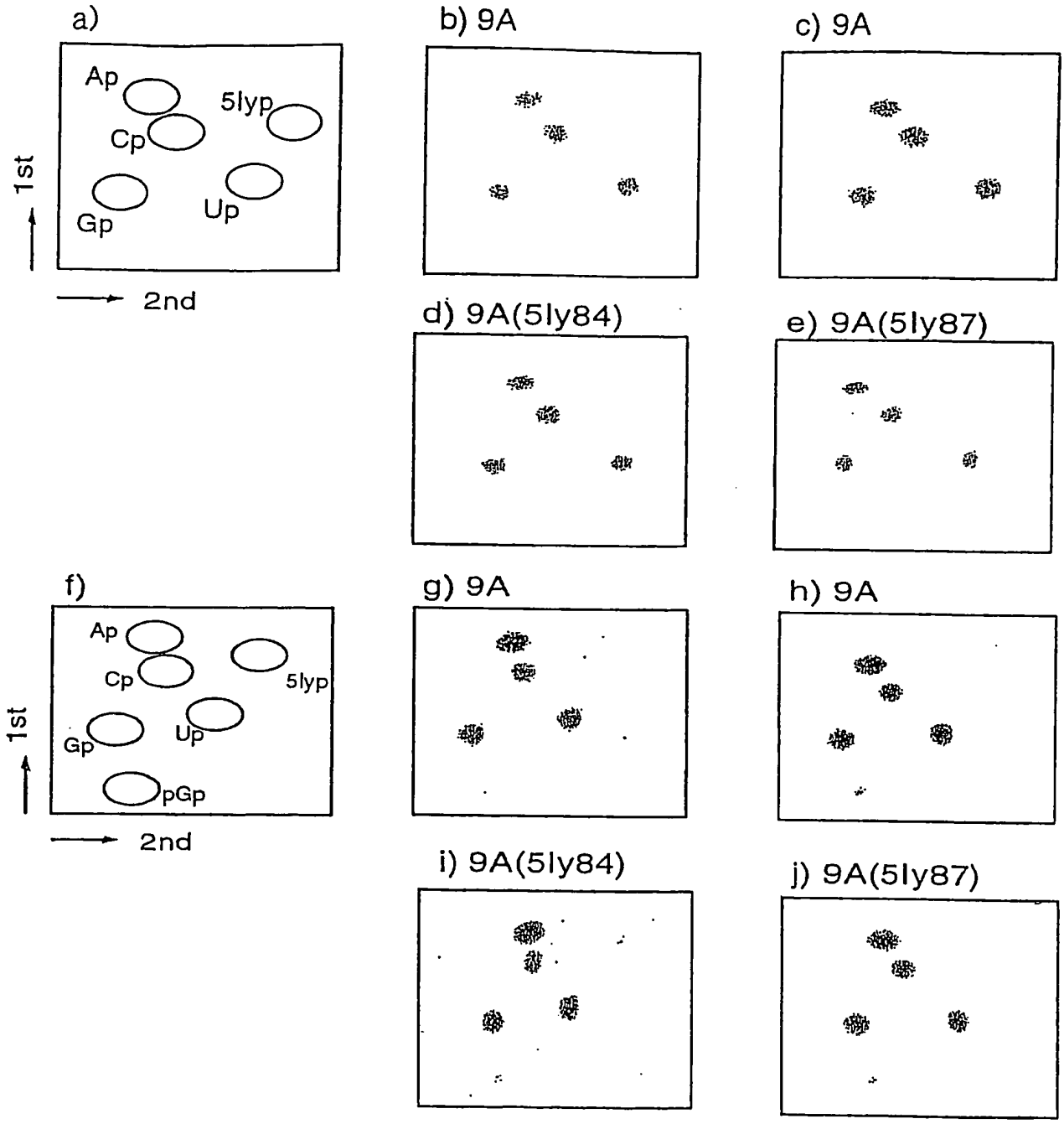

a)
Ap
5Iyp
Cp
Up
Gp
1st
2nd
b) 9A
c) 9A
d) 9A(5Iy84)
e) 9A(5Iy87)
f)
Ap
Cp
5Iyp
Gp
Up
pGp
1st
2nd
g) 9A
h) 9A
i) 9A(5Iy84)
j) 9A(5Iy87)

Figure 8

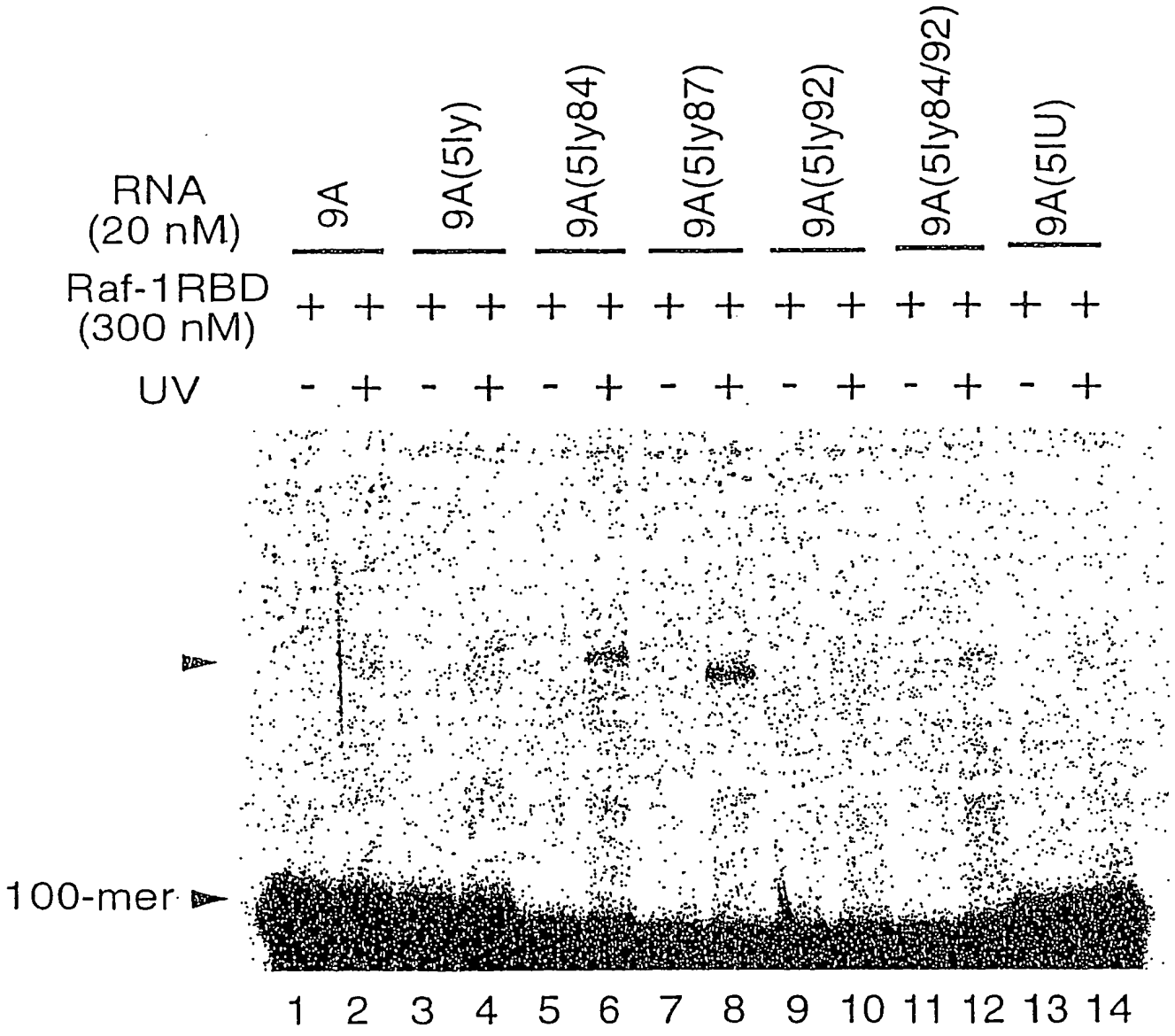

RNA (20 nM)
9A
9A(5Iy)
9A(5Iy84)
9A(5Iy87)
9A(5Iy92)
9A(5Iy84/92)
9A(5IU)
Raf-1RBD (300 nM)
UV
100-mer
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 9

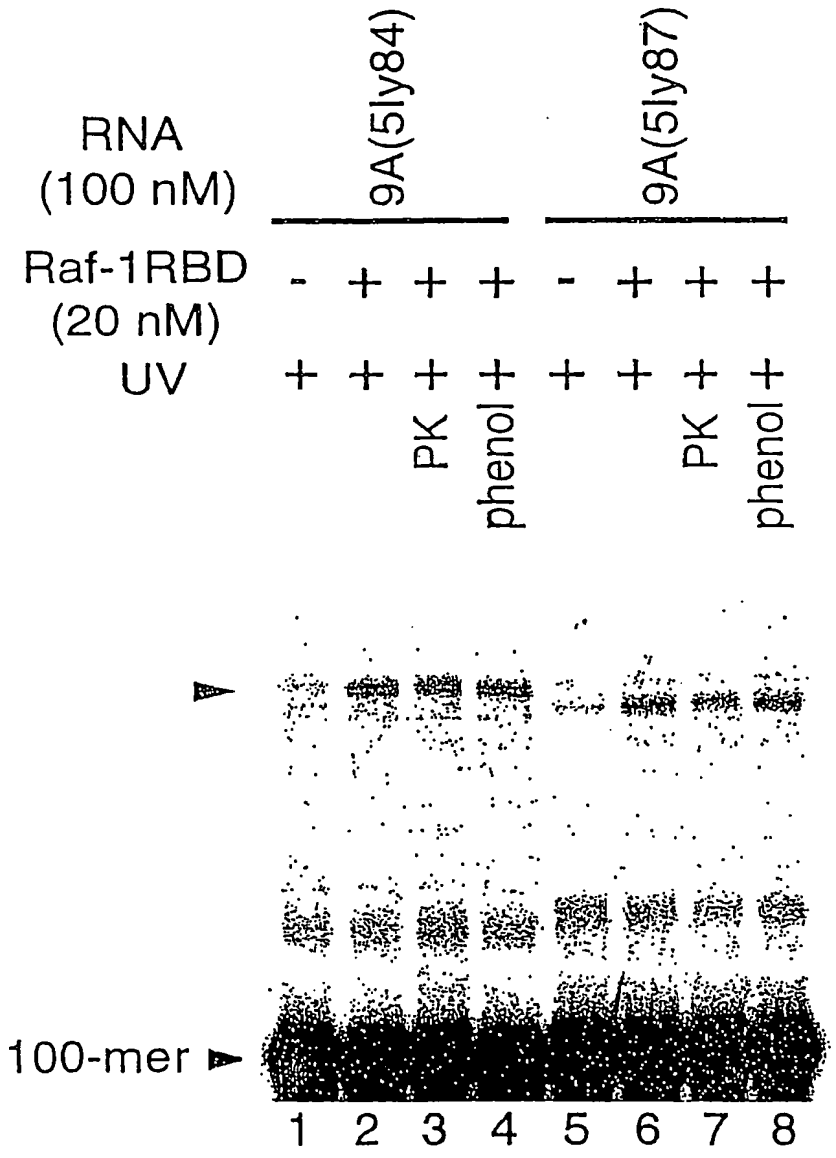
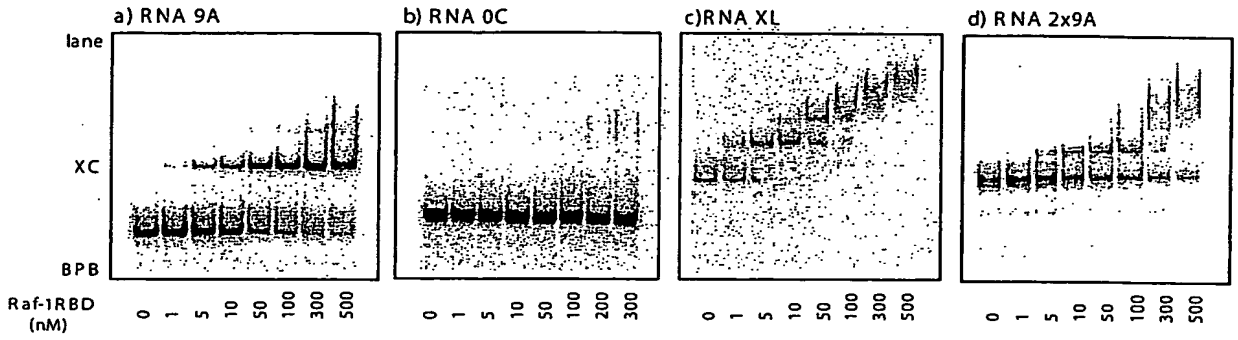

RNA
(100 nM)
9A(5ly84)
9A(5ly87)
Raf-1RBD
(20 nM)
UV
PK
phenol
PK
phenol
100-mer
1 2 3 4 5 6 7 8

Figure 10



e) RNA 9A :100-mer

5'-GGGAGUGGAGGAAUUCAUCGAGGCAU[-N45-]CAUAUGCCUUAGCGACAGCAAGCUUCUGC-3'

AUGUCGACUCCGUCUUCCUUCAAACCAGUUAUAAAUUGGUUUUAG

RNA 9A(5Iy87) :100-mer

5'-GGGAGUGGAGGAAUUCAUCGAGGCAU[-N45-]cauaugccuuagcga5IyCAGCAAGCUUCUGC-3'

RNA 2x9A :200-mer

5'-GGGAGUGGAGGAAUUCAUCGAGGCAU[-N45-]CAUAUGCCUUAGCGACAGCAAGCUUCUGC-

-GGGAGUGGAGGAAUUCAUCGAGGCAU[-N45-]CAUAUGCCUUAGCGACAGCAAGCUUCUGC-3'

RNA 0C :100-mer

5'-GGGAGUGGAGGAAUUCAUCGAGGCAU[-N45-]CAUAUGCCUUAGCGACAGCAAGCUUCUGC-3'

CUGGGAACCCUAUCUUGCUUUUGGUAGCUGUAUUCACCUGUAACAG

RNA XL : cross-linking product generated from two molecules of 9A(5Iy87)

Figure 11

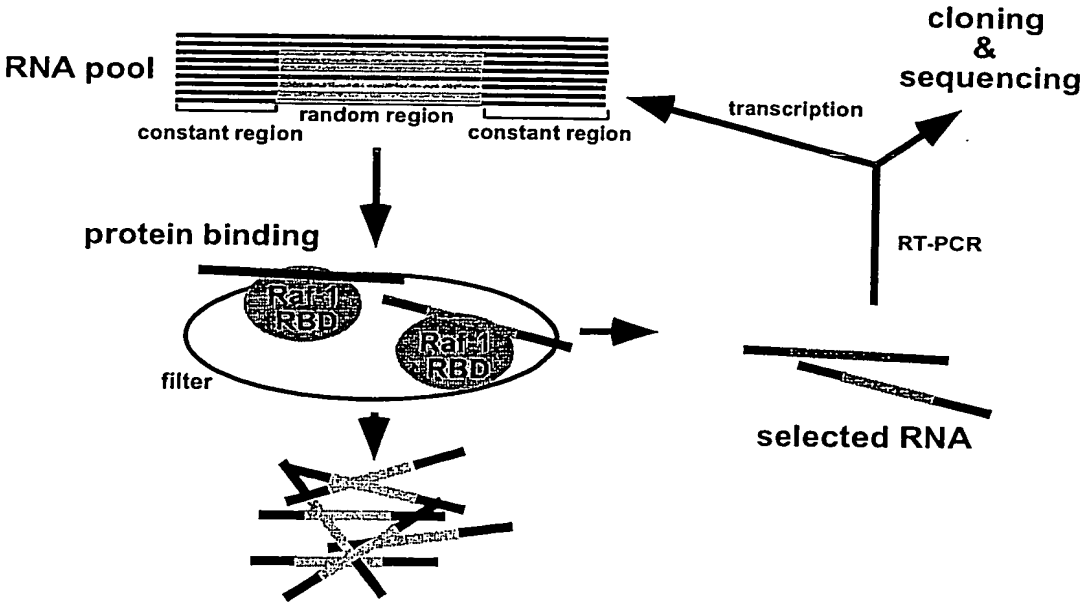

RNA pool
constant region
random region
constant region
transcription
cloning
&
sequencing
protein binding
RT-PCR
Raf-1
RBD
Raf-1
RBD
filter
selected RNA

Figure 12

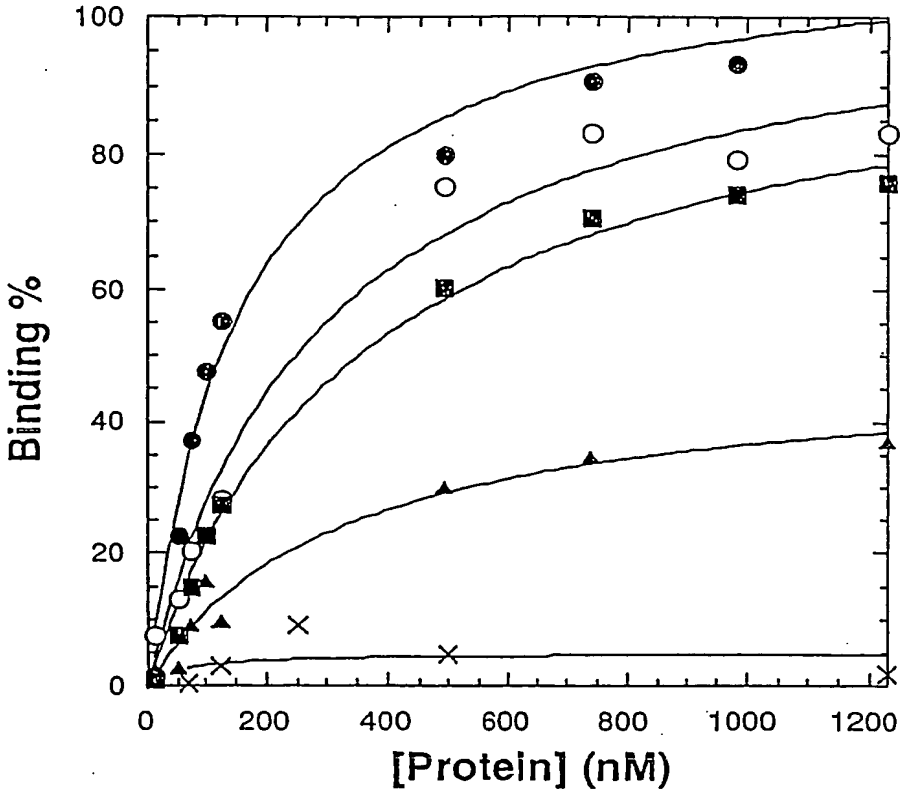


Figure 13

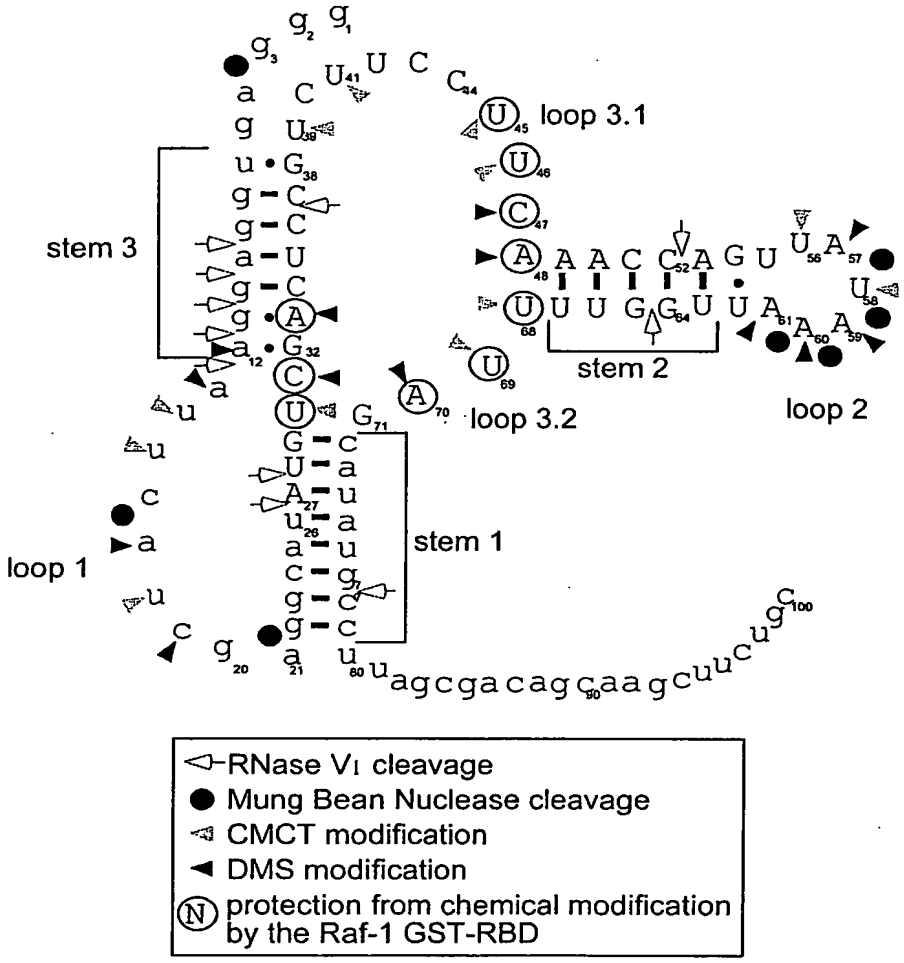

stem 3
loop 3.1
loop 3.2
loop 2
stem 2
stem 1
loop 1
RNase V1 cleavage
Mung Bean Nuclease cleavage
CMCT modification
DMS modification
protection from chemical modification by the Raf-1 GST-RBD

Figure 14

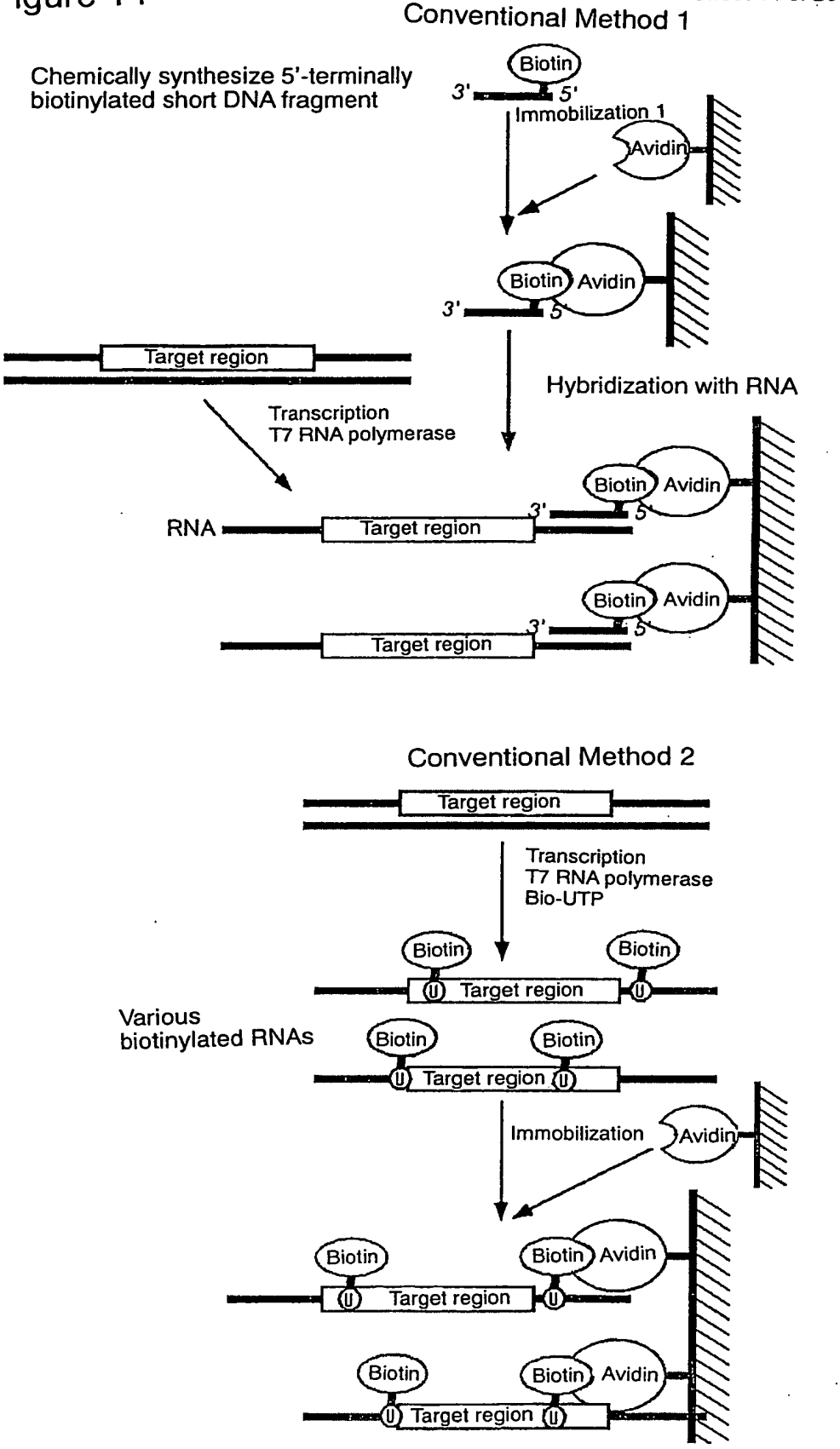
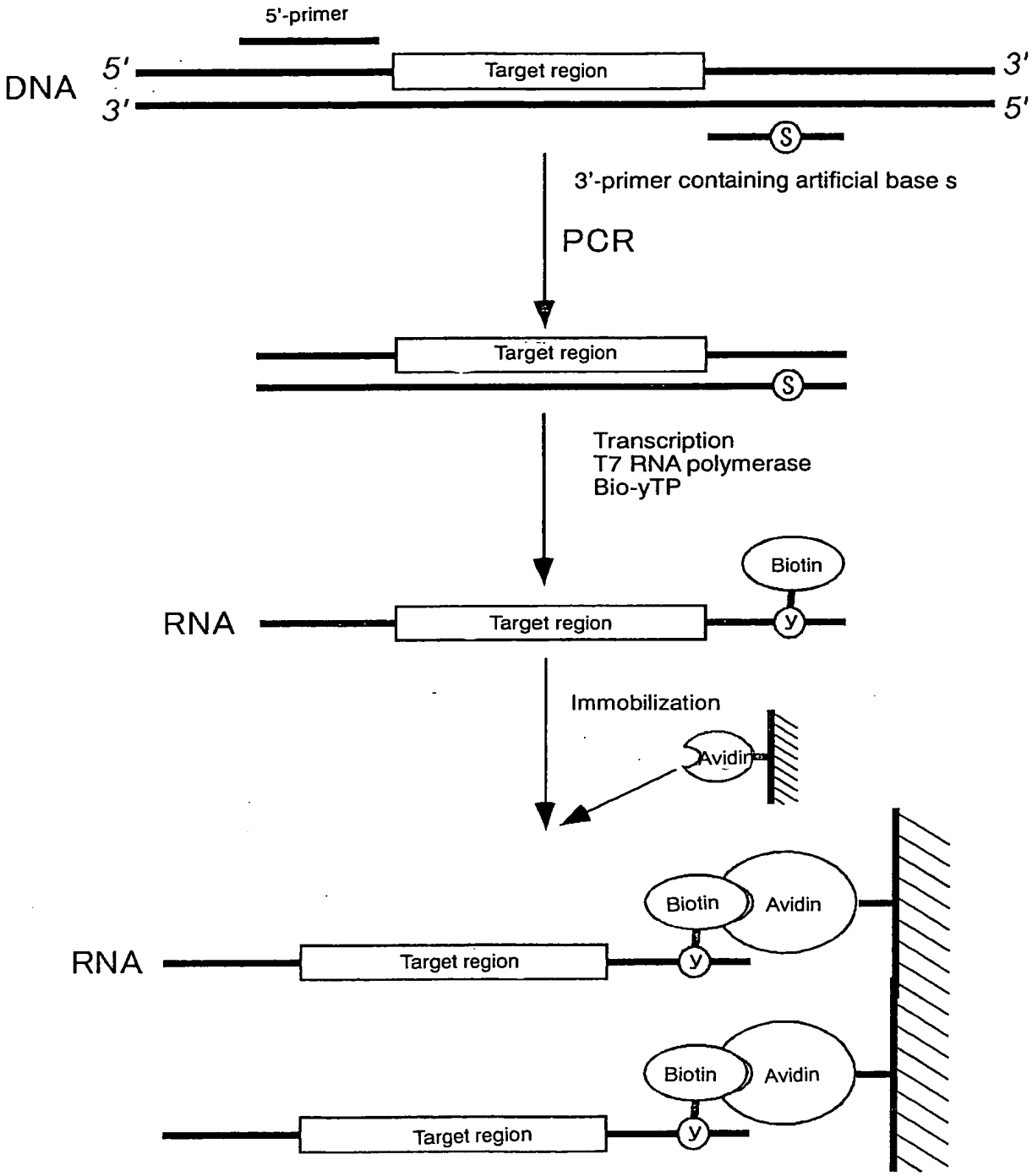

Conventional Method 1
Chemically synthesize 5'-terminally biotinylated short DNA fragment
Biotin
3'
5'
Immobilization 1
Avidin
Biotin
Avidin
3'
5'
Target region
Hybridization with RNA
Transcription
T7 RNA polymerase
Biotin
Avidin
3'
5'
RNA
Target region
Biotin
Avidin
3'
5'
Target region
Conventional Method 2
Target region
Transcription
T7 RNA polymerase
Bio-UTP
Biotin
Biotin
U
Target region
U
Various biotinylated RNAs
Biotin
Biotin
U
Target region
U
Immobilization
Avidin
Biotin
Biotin
Avidin
U
Target region
U
Biotin
Biotin
Avidin
U
Target region
U

Figure 14 (Continued)



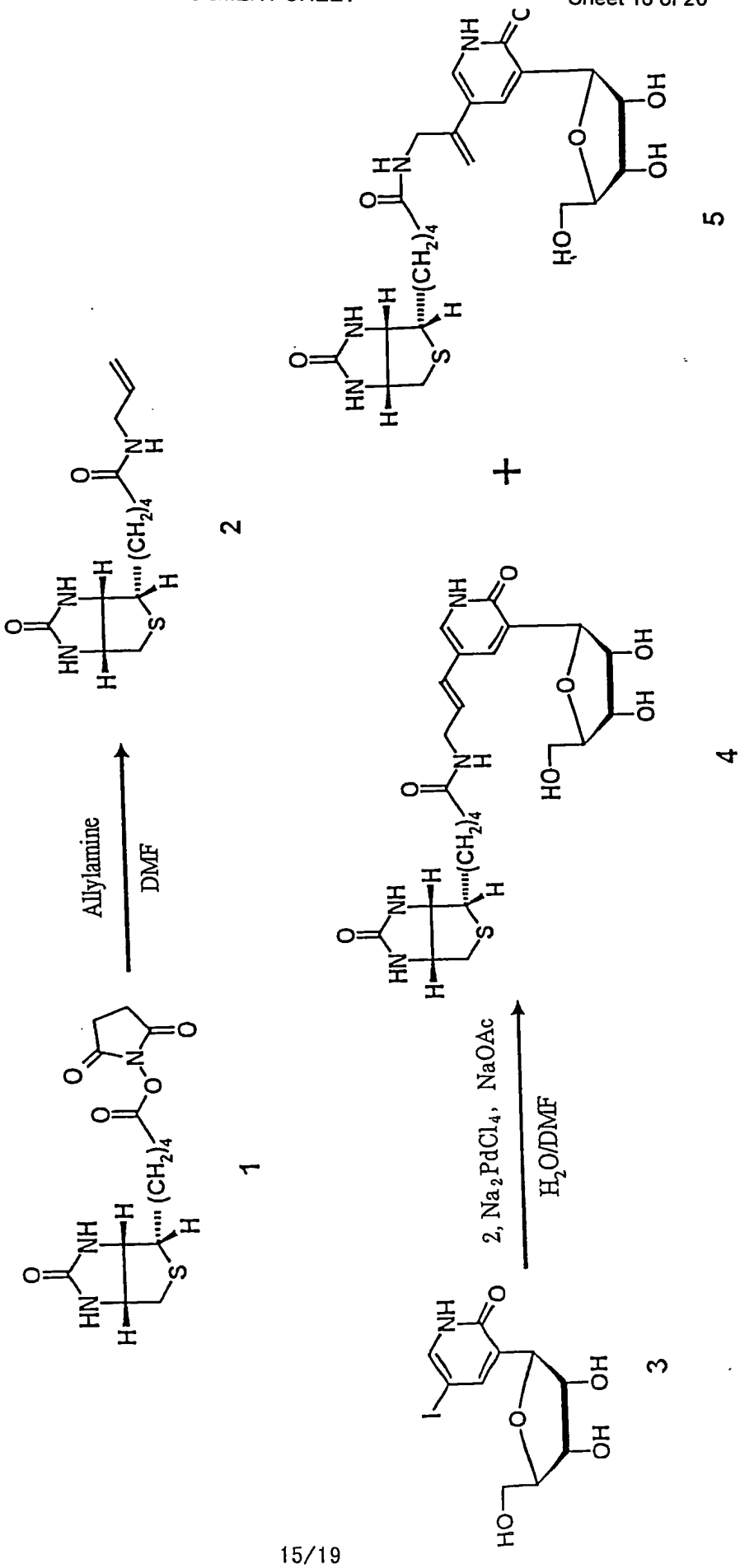


Figure 15

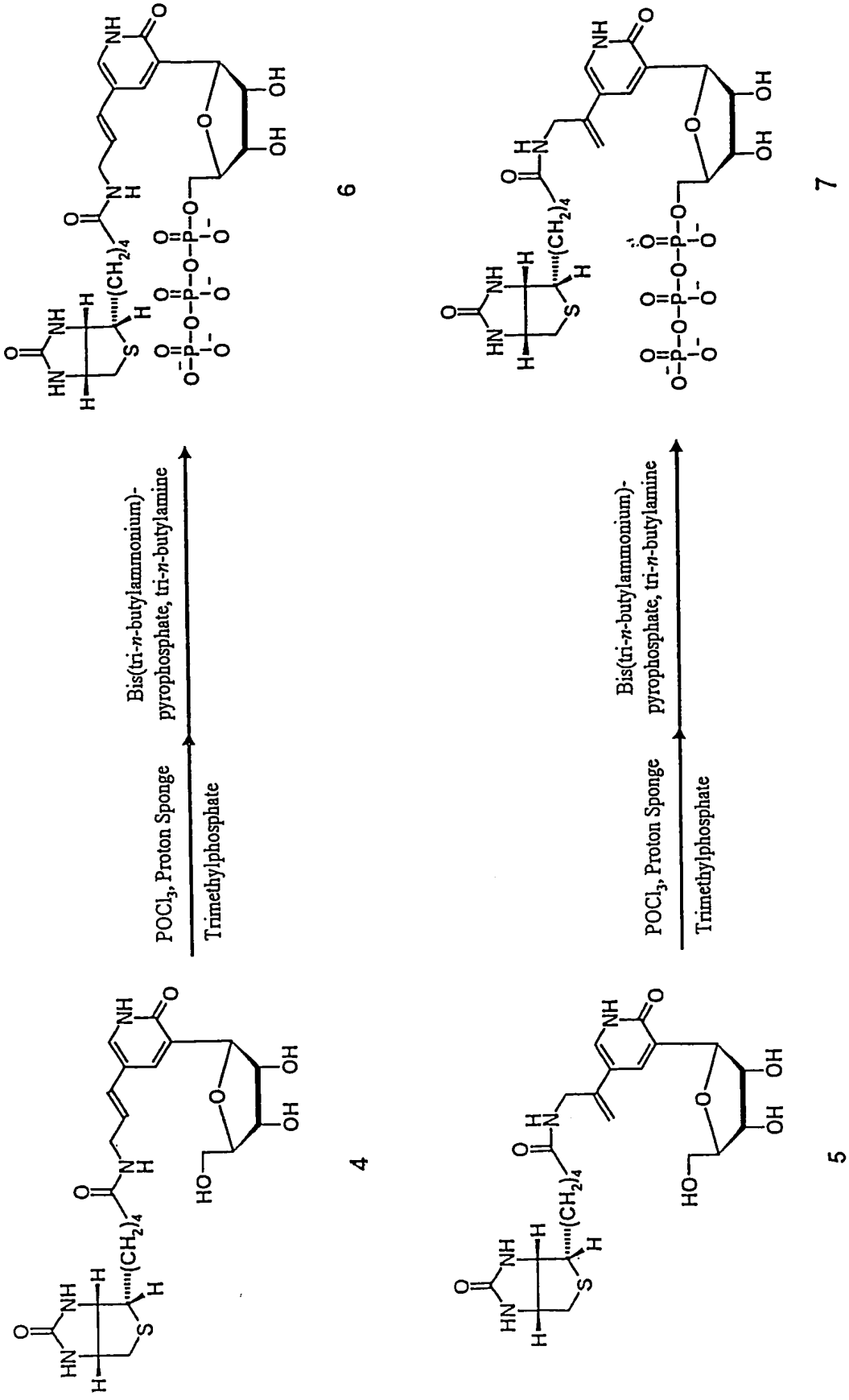

Bis(tri-n-butylammonium)-pyrophosphate, tri-n-butylamine
POCl3, Proton Sponge
Trimethylphosphate
Bis(tri-n-butylammonium)-pyrophosphate, tri-n-butylamine
POCl3, Proton Sponge
Trimethylphosphate
4
5
6
7

Figure 16

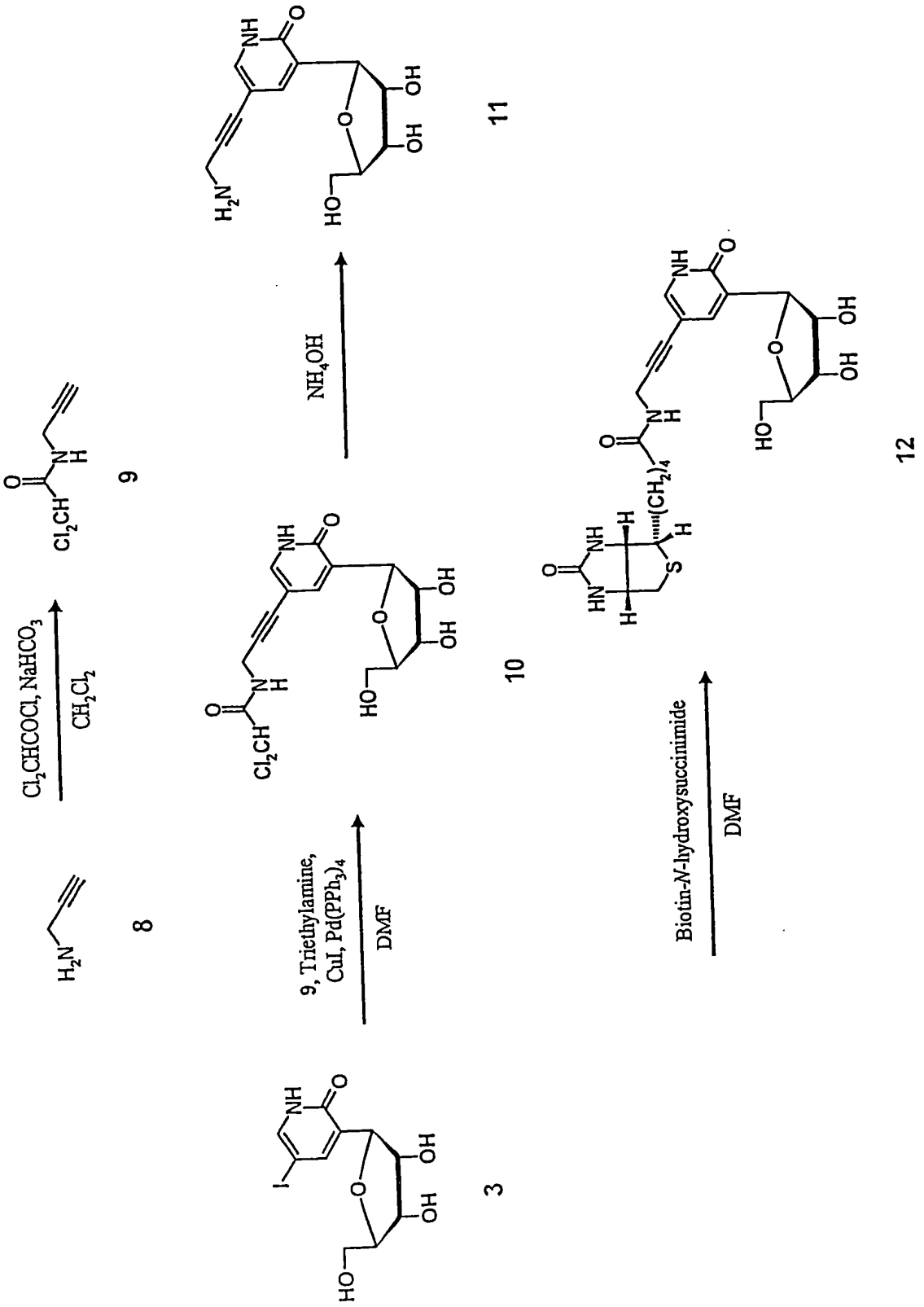
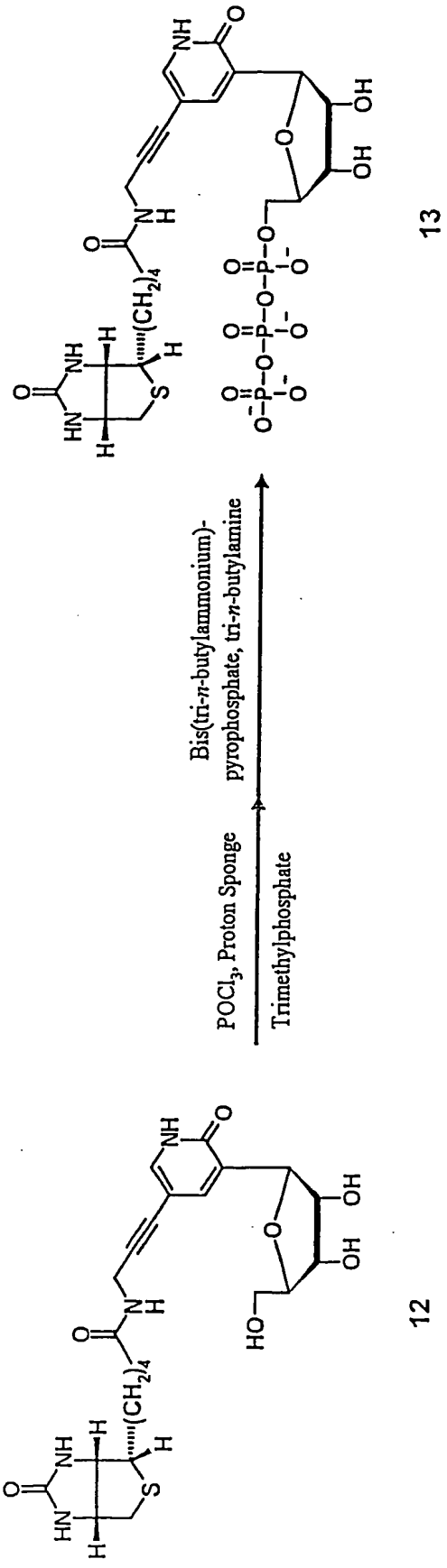

Cl2CHCOCl, NaHCO3
CH2Cl2
9, Triethylamine,
CuI, Pd(PPh3)4
DMF
NH4OH
Biotin-N-hydroxysuccinimide
DMF

Figure 17



13

12

Figure 18

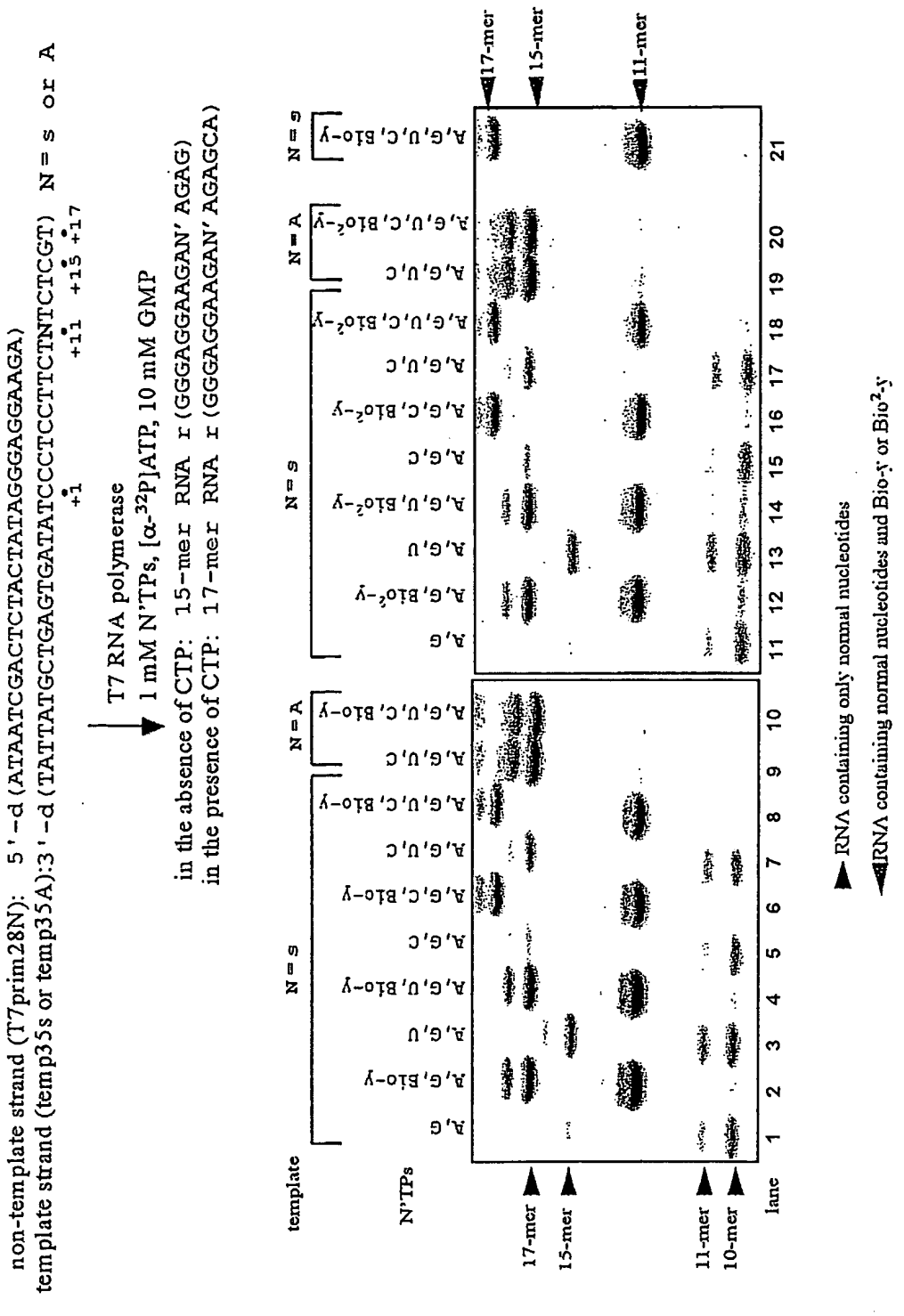

non-template strand (T7prim28N): 5'-d(ATAATCGACTCTACTATAGGGAGGAAGA)
template strand (temp35s or temp35A):3'-d(TATTATGCTGAGTGATATCCCTCCTTCTNTCTCGT) N = s or A
+1 +11 +15 +17
T7 RNA polymerase
1 mM N'TPs, [α-32P]ATP, 10 mM GMP
in the absence of CTP: 15-mer RNA r(GGGAGGAAGAN' AGAG)
in the presence of CTP: 17-mer RNA r(GGGAGGAAGAN' AGAGCA)
template
N = s
N = A
N = s
A,G
A,G,Bio-Y
A,G,U
A,G,U,Bio-Y
A,G,C
A,G,C,Bio-Y
A,G,U,C
A,G,U,C,Bio-Y
A,G,U,C
A,G,U,C,Bio²-Y
A,G
A,G,Bio²-Y
A,G,U
A,G,U,Bio²-Y
A,G,C
A,G,C,Bio²-Y
A,G,U,C
A,G,U,C,Bio²-Y
A,G,U,C
A,G,U,C,Bio-Y
N'TPs
lane
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
17-mer
15-mer
11-mer
10-mer
17-mer
15-mer
11-mer
RNA containing only normal nucleotides
RNA containing normal nucleotides and Bio-Y or Bio²-Y

Figure 19